Original Paper
ISSN: 2321-1520

Effect of Water Stress on the Alkaloid Content of *Catharanthes Roseus* L.

Dhruv Pandya*, Ancy Fernandez, Archana Mankad, Himanshu Pandya

Department of Botany, University School of Sciences, Gujarat University,Ahmedabad
E-mail: dhruvpandya1309@gmail.com
*Corresponding Author

Received Date: 5-2-2017
Published Date: 15-9-2017

Abstract

Catharanthes is an important herb used in traditional as well as herbal medicines. In this study an attempt is to made to study water stress and its effects on the alkaloid content in *Catharanthes roseus* L. The plants with different water stress conditions were used for the estimation. The alkaloid content was found to increase up to maximum 13.9% in water stressed plants as compared to the control.

Keywords: *Catharanthes roseus* L., water stress, alkaloid content, secondary metabolites.

Introduction

Catharanthes roseus L. which is an important medicinal plant of Apocynaceae is used to treat many of the fatal diseases, contains a virtual cornucopia of useful alkaloids, used in diabetes, blood pressure, asthma, constipation and cancer (M. Santhosh Aruna *et al*, 2015). Alkaloids are a diverse group of low molecular weight, nitrogen containing compounds found in about 20% plant species. Based on the strong evidence of biological activities of alkaloids, the study was focused on effect on water stress on the total alkaloid content using Harborne method.

Materials and Methods

Plant Material:

The plant material selected for the study was *Catharanthes roseus* L.and the saplings were purchased from Alpesh nursery, Shahibaug, Ahmedabad.

Methodology:

(A)Treatment to the plants:

The selected plants of *Catharanthes roseus L.* were grown in pots and given water stress condition in the Botanical Garden of Department of Botany,Gujarat University.The plants were grown under standard growing conditions and using standard cultural practices. They were divided in to four groups i.e., treated plants were watered once in every two, four and six days while one set was kept as control. Amount of water to all plants was 100ml.These treatments were continued up to four cycles for each set of plants.

(B)Collection and drying of plant material:

After the treatment of water stress condition to the plants for four cycles, all sets control, 2 day's treatment, 4 day's treatment and 6 day's treatment leaves were collected separately. The leaves were dried separately with the help of oven at 80^0C for 24 hours or till constant dry weight was achieved.

(C) Extract preparation method:

The dried leaves were weighed with the help of electric weighing balance and kept as separate packets. After weighing the leaves were crushed with the help of hands separately. Four beakers were selected and the crushed leaves powder was transferred into the beaker, and solvent ethanol was added in 1:10 ratio (one gram powder in 10ml ethanol). These processes were done for all sets including control, 2days, 4days and 6days. Covered the beakers with the help of aluminium foil, so solvent remains in the beakers and kept for 24 hrs. After that, filtered it with the help of whatmann filter paper no.1. Weighed four petri plates were selected and transfer the filtered liquid form in to the petri-plates. Then allowed to evaporate the solvent for 24 hours. After 24 hours again weighed the petri-plates with crude extract. The extract for all sets (control, 2days treated, 4days treated, 6days treated) was now used for estimations.

(D) Qualitative analysis of secondary metabolites:

(1) Alkaloids:

Mayer's test: 1ml filtrate was treated with 2ml Mayer's reagent; cream colour precipitation indicates the presence of alkaloids.

Wagner's test: 1ml filtrate was treated with Wagner's reagent; reddish brown colour indicates the presence of alkaloids.

Dragendorff's test: 1ml filtrate was treated with 2ml Dragendorff's reagent; orange red colour precipitation indicates the presence of alkaloids.

(2) Flavonoids:

Lead acetate test: 1ml liquid extract was treated with 10% lead acetate solution; formation of yellow precipitation indicates the presence of flavonoids.

H_2SO_4 test: 1ml extract was treated with few drops of H_2SO_4; orange colour precipitation indicates the presence of flavonoids.

Alkaline reagent test: 1ml of extract was treated with few drops of diluted NaOH and few drops of diluted HCl; yellow colour turns into colourless solution indicates the presence of flavonoids.

Zinc hydrochloride reduction test: 1ml extract was treated with zinc dust and concentrated HCl; formation of red colour indicates the presence of flavonoids.

Pew test: 1ml of extract was treated with pieces of metallic magnesium and 2-3 drops concentrated HCl were added; formation of brownish colour indicates the presence of flavonoids.

(3) Phenols:

Ferric chloride test: 1ml extract was treated with few drops of 5% ferric chloride solution; formation of bluish black colour indicates the presence of phenols.

Lead acetate test: 1ml extract was treated with 2-4 ml 10% acetic acid; formation of yellow colour precipitation indicates the presence of phenols.

(4) Saponins:

Frothing test: About 0.5 mg of extract was shaken with 5ml of distilled water; formation of froth (appearance of creamy small bubbles) shows the presence of saponins.

(5) Tannins:

Lead acetate test: 1ml of extract was treated with 1ml of 10% lead acetate solution; white colour precipitation indicates the presence of tannins.

Ferric chloride test: Small quantity of extract was mixed with water and heated in water-bath, the mixture was filtered and 0.1% ferric chloride solution was added to the filtrates; dark green colour indicates the presence of tannins.

Potassium dichromate test: 1 ml extract was treated with potassium dichromate; formation of dark precipitation indicates presence of tannins.

(6) Terpenoids:

Salkowski's test: Few mg of extract mixed with 2ml of Chloroform and 3ml of concentrated H_2SO_4 was carefully added to form a layer; an appearance of reddish brown colour ring indicates the presence of terpenoids.

Copper acetate test: Extract was dissolved in water and treated it with 5% copper acetate solution; formation of emerald green precipitation indicates the presence of terpenoids.

(7) Steroids:

Liebermann Burchard's test: 1ml of extract was treated with 2-3 ml acetic anhydrite solution and 2ml of sulphuric acid; violet or green colour indicates the presence of steroids.

Salkowski's test: 2mg of dry extract was shaken with chloroform; to the chloroform layer concentrated Sulphuric acid was added slowly by sides of test tubes; red colour indicates the presence of steroids.

(8) Glycosides:

Keller kiliani test: 2 ml of test solution was treated with few drops of glacial acetic acid and 1% ferric chloride solution mixed, concentrated Sulphuric acid was added and observed for the

formation of two layers; lower reddish brown and upper acetic acid layer which turns bluish green indicates a positive test for glycosides.

Bromine H_2O test: 1ml of test solution was dissolved in bromine water; formation of yellow colour precipitation indicates the presence of glycosides.

(9) Cardiac-glycosides:

Legal test**:** 2ml of test solution was treated with 1ml of pyridine and 1ml of 20% sodium nitroprusside; pink or red colour indicates the presence of cardiac-glycosides.

(E) Quantification of alkaloids:

Harborne method:

25mg of the sample was weighed in to a beaker, 50ml 10% acetic acid in ethanol was added and allow to stand for 4 hours. It was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added drop wise in to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitation was collected and washed with diluted NH_4OH and then filtered. The residue is the alkaloid, which was dried and weighed.

Formula for % Alkaloids: $\frac{W}{Y} \times 100$

Where W= Weighed of alkaloid content extracted

Y= Weight of plant material used.

Result and Discussions

(A) Qualitative analysis of secondary metabolites:

Result-table:1 of qualitative analysis of secondary metabolites in ethanolic leaves extract of *Catharanthes roseus L.*

As given in the table:

+ = present, - = absent

BIOCHEMICAL TESTS	Control	2 days	4 days	6 days
Alkaloids				
Dragendroff's test	+	+	+	+
Wagner's test	+	+	+	+
Mayer's test	+	+	+	+

Saponins				
Frothing test	-	-	-	-
Phenols				
Ferric chloride test	+	+	+	+
Lead acetate test	+	+	+	+
Flavanoids				
H_2SO_4 test	-	-	-	-
Lead acetate test	+	+	+	+
Alkaline test	-	-	-	-
Zinc hydrochloride test	-	-	-	-
Pew test	-	-	-	-
Tannins				
Lead acetate test	-	-	-	-
Ferric chloride test	-	-	-	-
Potassium dichromate test	+	+	+	+
Terpenoids				
Salkowski's test	+	+	+	+
Copper acetate test	+	+	+	+

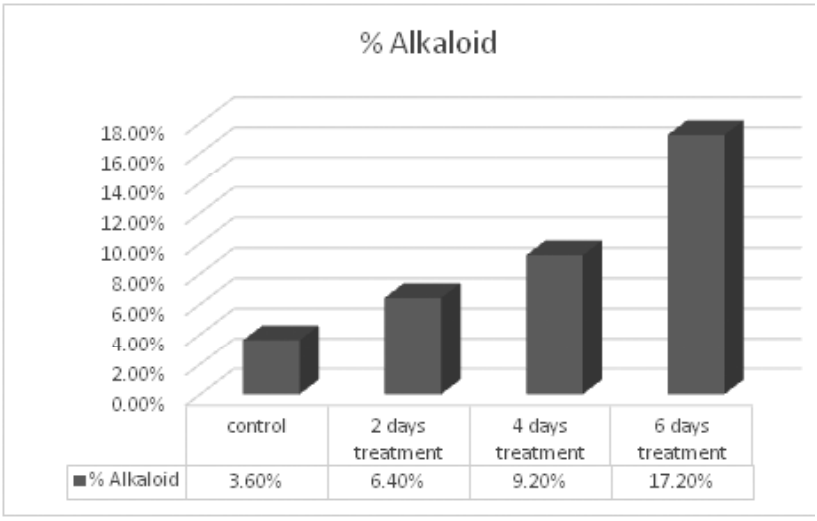
Steroids				
Liebermann burchard test	+	+	+	+
Salkowski's test	+	+	+	+
Glycosides				
Bromine water test	+	+	+	+
Keller kiliani test	+	+	+	+
Cardiac-glycosides				
Legal test	+	+	+	+

Table: 1 Qualitative analysis of secondary metabolites in ethanolic extract of *Catharanthes.*

(B) Quantification of alkaloid content:

In the quantification of alkaloids, as the water stress condition increased, the % alkaloids in leaves also increased (Fig 1). So, as compared to control % alkaloids increased in series of treatment were 2.8% in 2 day's treatment, 5.6% in four day's treatment, 13.9% in 6 day's treatment.

Fig 1 Showing amount of Alkaloids in samples due to water stress



Conclusion

Thus, the result obtained in the present study indicates *Catharanthes* leaves have the potential to act as a source of useful drugs because of presence of various phytochemical components such as alkaloids, phenols, terpenoids, steroids, glycosides. Harborne method established is appropriate for the quantification of alkaloids in percentage.

References

Favretto, D., Piovan, A., Filippini, R., & Caniato, R. (2001). Monitoring the production yields of vincristine and vinblastine in Catharanthus roseus from somatic embryogenesis. Semiquantitative determination by flow injection electrospray ionization mass spectrometry. *Rapid Communications in Mass Spectrometry*, *15*(5), 364-369.

Govindasamy, C., & Srinivasan, R. (2012). In vitro antibacterial activity and phytochemical analysis of Catharanthus roseus (Linn.) G. Don. *Asian Pacific Journal of Tropical Biomedicine*, *2*(1), S155-S158.

Haque, N., Chowdhury, S. A. R., Nutan, M. T. H., Rahman, G. M. S., Rahman, K. M., & Rashid, M. A. (2000). Evaluation of antitumor activity of some medicinal plants of Bangladesh by potato disk bioassay. *Fitoterapia*, *71*(5), 547-552.

Hedberg, I., Hedberg, O., Madati, P. J., Mshigeni, K. E., Mshiu, E. N., & Samuelsson, G. (1982). Inventory of plants used in traditional medicine in Tanzania. I. Plants of the families Acanthaceae-Cucurbitaceae. *Journal of ethnopharmacology*, *6*(1), 29-60.

Karthik, D., & Ravikumar, S. (2011). Proteome and phytochemical analysis of Cynodon dactylon leaves extract and its biological activity in diabetic rats. *Biomedicine & Preventive Nutrition*, *1*(1), 49-56.

Kabesh, K., Senthil Kumar, P., Ragunathan, R., & Kumar, R. R. (2015). Phytochemical analysis of Catharanthus roseus Plant Extract and its Antimicrobial Activity. *Int. J. Pure App. Biosci*, *3*(2), 162-172.

Nain, P., Saini, V., Sharma, S., & Nain, J. (2012). Antidiabetic and antioxidant potential of Emblica officinalis Gaertn. leaves extract in streptozotocin-induced type-2 diabetes mellitus (T2DM) rats. *Journal of Ethnopharmacology*, *142*(1), 65-71.

Ohadoma, S. C., & Michael, H. U. (2011). Effects of co—administration of methanol leaf extract of Catharanthus roseus on the hypoglycemic activity of metformin and glibenclamide in rats. *Asian Pacific journal of tropical medicine*, *4*(6), 475-477.

Roepke, J., Salim, V., Wu, M., Thamm, A. M., Murata, J., Ploss, K., ... & De Luca, V. (2010). Vinca drug components accumulate exclusively in leaf exudates of Madagascar periwinkle. *Proceedings of the National Academy of Sciences*, *107*(34), 15287-15292.

Subbaiyan, B., Samydurai, P., KarthikPrabu, M., Ramakrishnan, R., & Thangapandian, V. (2013). Physico-phytochemical analysis and antibacterial prospective of Catharanthus pusillus (Murray) G. Don an important anticancer medicinal plant. *Int J Pharm Pharm Sci*, *5*(2), 212-215.

Spring, O. (2000). Chemotaxonomy based on metabolites from glandular trichomes. *Advances in Botanical research*, *31*, 153-174.

Yasmeen, M., Prabhu, B., & Agashikar, N. V. (2010). Evaluation of the antidiarrhoeal activity of the leaves of Ixora coccinea Linn. in rats. *J Clin Diagn Res*, *4*(5), 3298-3303.